Structure for Electro-Spun Silk Fibroin Nanofibers

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ABSTRACT: Electro-spinning was made using silk fibroin and it was found that silk fibroin nanofibers of partially oriented amorphous structure are producible using HFIP(1,1,1,3,3,3 hexafluoro-2-propanol) as a solvent for fibroin via the electro-spinning setup equipped with parallel electrodes as a collector. Transformation from amorphous to silk I of a highly contracted beta-turn form or amorphous to silk II of a regular array of antiparallel beta-sheets occurred preferentially via the treatment with water vapor or ethanol, respectively. In addition the c-axis of crystallites was oriented parallel to the fiber axis. When the electro-spinning was made using a dish-type collector filled with ethanol, a peculiar web texture was obtained. Such a web texture seems to be brought about by the shrinkage of fiber due to the crystallization of fibroin and/or surface tension of ethanol droplets formed between the fibers. In this spinning setup, the c-axis of crystallites was also oriented parallel to the fiber axis. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 3681–3684, 2008

Key words: silk fibroin; electro-spinning; nanofiber; structure; morphology

INTRODUCTION

In addition to the utility of the silk fibroin as textile fiber, silk fibroin was proved to be an ideal material for biomedical applications.[1] To produce a functional biomedical product from silk fibroin, the development of new processing technology is needed. In the field of tissue engineering, increasing attention has been paid on the electro-spinning system that can supply nanofibers.[2] Also, in the case of silk fibroin, it has been reported that the number of human bone-marrow stromal cells attaching on the silk fibroin films consisting predominantly of silk I crystals is significantly higher than the methanol-treated films having the surface rich in silk II structure.[3] There are several reports dealing with the production of nanofibers from silk fibroin, for example, Ko and coworkers reported the processing parameters and geometric properties for the regeneration of *B. mori* silk fibroin by electro-spinning.[4] Asakura and coworkers tried to produce non-woven nanofibers of hybrid silk by electro-spinning and investigated the structural changes during the process of the fiber formation using NMR spectroscopy.[5] However, crystal morphologies and/or fiber structure of regenerated silk fibroin nanofibers have not been fully discussed. Silk fibroin shows three crystal modifications, i.e., silk I of a highly contracted beta-turn form,[6,7] silk II of a regular array of antiparallel beta-sheets,[8] and silk III of threefold helical form.[9] To control the crystal structure of nanofibers, it is preferable to separate the spinning process and the annealing process. First of all in the spinning process it is necessary to produce amorphous nanofibers. Then the as-spun fibers are followed by the annealing treatment to generate and control the crystal structure.

In the present research, silk fibroin nanofibers were spun by electro-spinning and their structure was controlled by changing the geometrical configuration of a conductive collector and the annealing conditions using water or ethanol.

EXPERIMENTAL

Materials

B.mori silk fibroin obtained through the degumming of cocoons in high pressurized water at 120°C for 1 h was dissolved in a ternary solvent system of $CaCl_2/CH_3CH_2OH/H_2O$ (1/2/8 in mole ratio) at 90°C for 2 min and the solution was separated from any foreign particles by centrifugation. A regenerated fibroin solution was obtained by filtering the centrifuged solution and by dialyzing the filtrate for 3 days using cellulose tubes at 25°C. Then the solution was frozen-dried and silk fibroin amorphous gel was prepared. The gel was dissolved in 1,1,1,3,3,3 hexafluoro-2-propanol (HFIP) and the solution of 5 wt % was prepared for the spinning.

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Asakura and coworkers[5] used hexafluoroacetone-hydrate (HFA) as a solvent for silk fibroin. However, the additional treatment with methanol was needed to remove HFA completely from regenerated fibers. In addition, the methanol treatment tends to promote the so-called solvent-induced crystallization and most stable silk II crystals are formed. Ko and coworkers[4] used formic acid instead. In their case, solvent-induced crystallization also occurs and stable silk II crystals are formed. To investigate the crystal modifications of silk fibroin nanofibers, it is important to produce amorphous precursors by electro-spinning. When silk fibroin is dissolved in HFIP, the presence of an ordered secondary structure 3_{10}-helix has been confirmed from the high-resolution solution ^{13}C-NMR and CD studies.[10] Such structure will be advantageous to prevent the generation of most stable silk II crystals in the course of spinning line and will be useful to produce the amorphous nanofibers.

Electro-spinning

Electro-spinning was carried out with an ''esprayer ES-1000'' (Fuence Co., Ltd., Tokyo, Japan). The solution was loaded into a glass syringe having a needle made of stainless steel. The needle was connected to a high-voltage supply. The solution was continuously supplied using a syringe pump at a rate of 20 μL/min through the needle. A voltage of 20 kV was applied for electro-spinning. The distance between the needle tip and the collector was ~ 10 cm. Li and Xia[2] demonstrated that electro-spun fibers could be aligned in parallel over long-length scales during the spinning process by using a collector consisting of two conductive strips separated by a void gap of variable widths up to several centimeters. Thus we designed a collector consisting of two parallel metal plates instead of a normal single metal plate collector.[11] The gap between the plates was ~ 5 mm. An aluminum dish of ~ 6 cm in diameter filled with ethanol was also used as a collector.

TEM observation

Selected-area electron diffraction (SAED) of resulting specimens was performed at room temperature with a TEM (JEOL JEM-200CS) operated at an accelerating voltage of 200 kV. The nanofibers were mounted on aluminum-coated copper grids to estimate the camera length and determine the diffraction angle. The diameter of selected area on the grid was ~ 2.5 μm.

RESULTS AND DISCUSSION

Figure 1 is a polarized light micrograph observed for the as-spun silk fibroin fibers using the parallel-type collector. In this geometrical configuration of a collector, the fibers will experience a strong stretching force due to the Coulombic interactions between the positive charges on the fiber and the negative imaginary charges on the two grounded electrodes.[2] Thereby the electro-spun fibers were aligned in parallel spanning across the gap between the two electrodes. Figure 1 also suggests that the as-spun fibers had a partially oriented structure. It is well known that electro-spinning involves the rapid stretching of an electrical jet. Thus an elongational stress will probably be applied to fibroin molecules in the solution. Therefore, fibroin molecular chains were stretched and partially oriented along the fiber axis. We confirmed similar behavior in the electro-spinning of polydioxanone.[11] Rabolt and coworkers[12] have reported that electro-spinning of Nylon-6 led to formation of the γ-form rather than the α-form that was observed in films cast from solutions. Moreover, no crystal reflection was observed in the SAED pattern for the as-spun fibers of silk fibroin. Therefore, the obtained fibers had a partially oriented amorphous structure.

Figure 1 Polarized light micrograph for the silk fibroin fibers electro-spun using the parallel-type collector. The scale bar corresponds to 30 μm.

Figure 2 shows a SAED pattern for the silk fibroin nanofiber electro-spun using the parallel-type collector and exposed to water vapor at 25°C for 24 h. Silk fibroin was crystallized via the water vapor annealing and silk I crystals[5,6] were obtained preferentially. Similar crystallization behavior was observed in a case of silk fibroin film.[13] Furthermore the reflection 110 from crystallites concentrated on the equator. Kaplan and coworkers[3] used water-annealing instead of water vapor to obtain silk I crystals. However amorphous silk fibroin nanofibers will readily dissolve in water. In order to produce silk fibroin nanofibers of silk II crystals, the fibers electro-spun using the parallel-type collector were treated in ethanol at 25°C for 1 min. In this case the overlapped reflections of 200, 020, 210, 120[8] from crystallites also concentrated on the equator.

When the electro-spinning was made using a normal flat collector, SAED patterns of as-spun fibers

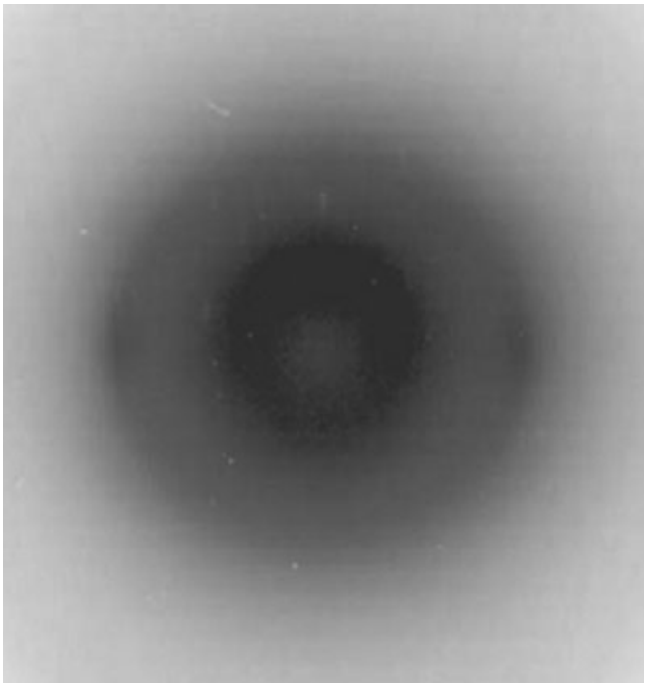

Figure 2 SAED pattern of silk fibroin nanofibers electro-spun using the parallel-type collector. The fiber axis is vertical.

showed only amorphous halos, and then reflections from crystallites did not concentrate on an equator even after the fibers were crystallized via the treatment with water vapor or ethanol.

The difference in SAED patterns between the electro-spinning geometrical setups using the parallel-type collector and the normal flat collector suggests that the relaxation of silk fibroin molecules occurred after the fibers were collected on the collectors, that is, the evaporation of the solvent was not fully completed on the spinning line under the conditions used in this study. When the two spinning setups are compared from the viewpoint of the relaxation of molecular orientation in fiber, SAED patterns indicate that the spinning system using parallel electrodes is more advantageous to suppress the relaxation of molecular orientation and more suitable to capture the fiber structure formed in the course of spinning. In this setup a stretching force generated by the Coulombic interactions between the fiber and the electrodes seems to promote the release of solvent. Therefore the SAED pattern in Figure 2 shows a fiber diagram. The enhancement in molecular orientation is important to produce flexible and ductile regenerated silk fibroin fibers.

When an aluminum dish filled with ethanol was used as a collector, the fibers were collected spanning across the arbitrary two points on the edge of dish and finally the dish was completely covered with the collected non-woven electro-spun web. When compared with a normal flat metal collector, the web can be easily removed from the collector due to the space between the web and liquid surface, and then the flat web can be produced without breaking.

The dish was filled with ethanol, and accordingly the fibers were exposed to ethanol vapor. SAED measurements revealed that silk II crystals were formed preferentially, and in addition the c-axis of crystallites was oriented parallel to the fiber axis (see Fig. 3). From the comparison of the azimuthal widths for the diffractions on equator in Figures 2 and 3, the diffraction peaks in Figure 3 is slightly wider. Thus it seems that the degree of orientation for the nanofibers produced using the dish-type collector is not so marked as compared to the case using the parallel-type one.

When the fibers are collected spanning across the arbitrary two points on the edge of dish, the relaxation of molecular orientation in fiber will possibly be suppressed to a certain extent by a stretching force similarly in the case of the parallel-type collector. As for the spinning setup using a dish-type collector, however, the distances between bridging points are much larger as compared with the setup equipped with the parallel-type collector. The diameter of the dish is $\sim$ 6 cm. Thus the collected fiber will tend to bend due to the relaxation of molecular orientation especially if the fiber formation is not completed. Figure 4 is a scanning electron micrograph of electro-spun web prepared using a dish-type collector. The remarkable bending of fibers can be recognized. When compared with the crystal formation of silk I in the annealing using water vapor, the crystal formation of silk II by the annealing with ethanol proceeds easily. Accordingly the relaxation of molecular

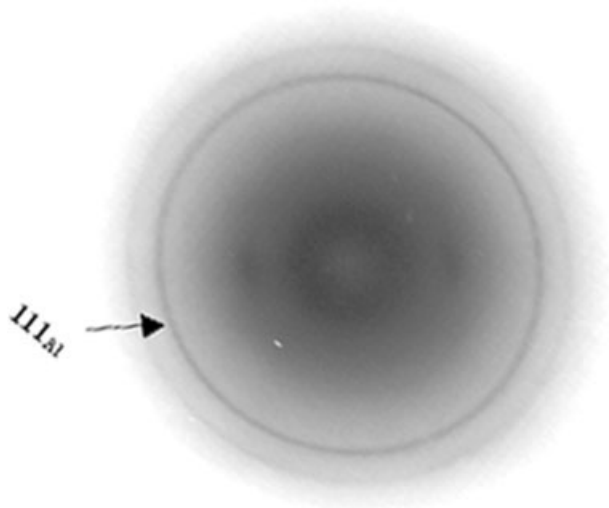

Figure 3 SAED pattern for the fibers electro-spun using the dish-type collector. The fiber axis is vertical.

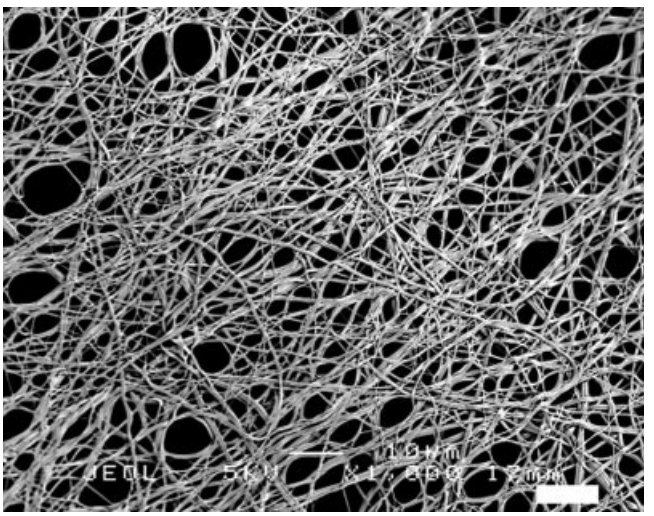

Figure 4 Scanning electron micrograph of silk fibroin fibers electro-spun using an aluminum dish filled with ethanol as a collector. The scale bar corresponds to 10 μm.

orientation in fiber was rather suppressed due to the solvent induced crystallization brought about by ethanol in the dish-type collector. Therefore, Figure 3 showed a fiber diagram similar to that in Figure 2.

In addition, when the electro-spinning was made using a dish-type collector the fibers were randomly bundled or entangled (see Fig. 4). Such a web texture was not observed in the electro-spinning using a normal single metal plate collector.

In general, the fibers tend to shrink when crystallization occurs in the amorphous regions. The crystallization rate is influenced by the molecular orientation of fibroin chain, the diffusion of ethanol into the fiber, and so on. Thus, the crystallization rate became different in every portion of fiber due to the irregularity in fiber diameter. Hence, crystallization tends to proceed irregularly in the web. The random shrinking of fibers in the web due to the crystallization possibly caused such a peculiar web texture.

Another possible mechanism to produce the bundles or entanglements of fibers in web depends on the surface tension of liquid in the dish-type collector. When the successive procedure of dipping into liquid and drying in air is made on as-spun fibers, the fibers are randomly bundled by the droplets of liquid. Therefore, it is possible that the randomly formed droplets of ethanol between the fibers caused the entanglements. This mechanism will be useful to increase the thickness of web and produce a three-dimensional fibrous structure through controlling the surface tension of liquid properly and balancing shrinkage and drying rate for the web.

CONCLUSIONS

Silk fibroin nanofibers with having partially oriented amorphous structure are producible using HFIP as a solvent for fibroin via the electro-spinning setup equipped with parallel electrodes as a collector. Transformation from amorphous to silk I of a highly contracted beta-turn form or amorphous to silk II of a regular array of antiparallel beta-sheets occurred preferentially via the treatment with water vapor or ethanol, respectively. In addition the c-axis of crystallites was oriented parallel to the fiber axis.

When the electro-spinning was made using a dish-type collector filled with ethanol, a peculiar web texture was obtained. When compared with a normal flat metal collector, the web can be easily removed from the collector because of the space between the web and liquid surface, and then the flat web can be produced without breaking. In this spinning setup, the c-axis of crystallites was also oriented parallel to the fiber axis. A dish-type collector will be useful to increase the thickness of web, and then a three-dimensional silk fibroin material will be producible by controlling the surface tension of liquid properly and balancing shrinkage and drying rate for the web. A three-dimensional silk fibroin nanofibrous structure will be useful to impregnate living cells into the material and to produce the artificial tissue.

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